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Research Report

Antibody-mediated targeted gene transfer of helper virus-free HSV-1 vectors to rat neocortical neurons that contain either NMDA receptor 2B or 2A subunits

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ABSTRACT

Because of the numerous types of neurons in the brain, and particularly the forebrain, neuron type-specific expression will benefit many potential applications of direct gene transfer. The two most promising approaches for achieving neuron type-specific expression are targeted gene transfer to a specific type of neuron and using a neuron type-specific promoter. We previously developed antibody-mediated targeted gene transfer with Herpes Simplex Virus (HSV-1) vectors by modifying glycoprotein C (gC) to replace the heparin binding domain, which mediates the initial binding of HSV-1 particles to many cell types, with the Staphylococcus A protein ZZ domain, which binds immunoglobulin (Ig) G. We showed that a chimeric gC–ZZ protein is incorporated into vector particles and binds IgG. As a proof-of-principle for antibody-mediated targeted gene transfer, we isolated complexes of these vector particles and an anti-NMDA NR1 subunit antibody, and demonstrated targeted gene transfer to neocortical cells that contain NR1 subunits. However, because most forebrain neurons contain NR1, we obtained only a modest increase in the specificity of gene transfer, and this targeting specificity is of limited utility for physiological experiments. Here, we report efficient antibody-mediated targeted gene transfer to NMDA NR2B- or NR2A-containing cells in rat postrhinal cortex, and a neuron-specific promoter further restricted recombinant expression to neurons. Of note, because NR2A-containing neurons are relatively rare, these results show that antibody-mediated targeted gene transfer with HSV-1 vectors containing neuron type-specific promoters can restrict recombinant expression to specific types of forebrain neurons of physiological significance.

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1. Introduction

Given the complex cellular composition of the brain, and especially the forebrain, neuron type-specific recombinant gene expression is required for many potential uses of direct gene transfer into neurons. The two primary approaches for obtaining neuron type-specific expression are modifying a virus vector particle protein for targeted gene transfer to a specific type of neuron or use of a neuron type-specific promoter (Kasahara et al., 1994; Muller et al., 2003; Rasmussen et al., 2007; Song et al., 1997; Wang et al., 2005; Wickham, 2003; Wickham et al., 1996a). Importantly, targeted gene transfer supports

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efficient neuron type-specific expression by reducing the background of gene transfer to undesirable neuron types. Of note, these two approaches are complementary, and more restricted specificities of expression cay be obtained by using both of these approaches. Thus, targeting gene transfer to cells that contain specific NMDA receptor subunits, in combination with a neuron-specific promoter, could selectively support expression in neurons that contain specific NMDA receptor subunits. This specificity in expression would have multiple uses in neural gene transfer studies for gene therapy or basic neuroscience.

Targeted gene transfer has been developed using classical retrovirus, lentivirus, adeno-associated virus (AAV), adenovirus, and Herpes Simplex Virus (HSV-1) vectors (Buning et al., 2003; Cao et al., 2008, 2010; Douglas et al., 1996; Grandi et al., 2004; Kasahara et al., 1994; Laquerre et al., 1998a; Peng and Russell, 1999; Wang et al., 2005; Wickham, 2003; Wickham et al., 1996a,b). The most direct targeting strategy is to modify a vector particle protein to add a specific binding capability, but a limitation of this strategy is that it is specific for a particular ligand. A more general targeting strategy is to modify a vector particle to bind an antibody. This strategy theoretically supports targeting to any cell surface epitope for which an antibody exists, or can be derived. Antibody-mediated targeted gene transfer has been developed by modifying a specific vector particle protein to contain the Staphylococcus A protein ZZ domain, an immunoglobulin (Ig) G binding domain. This strategy has been used to target classical retrovirus, lentivirus, AAV, adenovirus, and sindbis virus vectors to specific peripheral cell types (Bergman et al., 2003; Morizono and Chen, 2005; Morizono et al., 2001, 2005; Ohno et al., 1997; Ried et al., 2002; Tai et al., 2003; Volpers et al., 2003), and to target HSV-1 vectors to a specific cell type in the brain (Cao et al., 2010).

Helper virus-free HSV-1 plasmid (amplicon) vectors have desirable properties and can support both targeted gene transfer and use of neuron-specific promoters. These vectors have a large capacity and efficiently transduce neurons (Fraefel et al., 1996; Geller and Breakefield, 1988; Geller et al., 1991). Of note, long-term, neuron-specific expression in forebrain areas is supported by HSV-1 vectors that contain a modified neurofilament heavy gene promoter (Sun et al., 2004; Zhang et al., 2005). Importantly, targeted gene transfer is based on the entry mechanism for wt HSV-1: HSV-1 particle entry is mediated by the outermost layer of a HSV-1 particle, the envelope, a lipid bilayer containing ~10 viral-encoded glycoproteins (Roizman and Sears, 1993), and entry requires specific sequential steps (Spear and Longnecker, 2003). Initial binding to glycosaminoglycans, primarily heparin sulfate, on cell surface proteoglycans is mediated by HSV-1 glycoprotein C (gC) and gB (Laquerre et al., 1998b; Mardberg et al., 2001; Shukla and Spear, 2001; Tal-Singer et al., 1995). Next, gD binds to specific receptors, and most cells contain at least one receptor for HSV-1 (Spear and Longnecker, 2003). Entry occurs by HSV-1 envelope fusion to the cell membrane and requires gB, gD, gH, and gL. The first studies on targeted gene transfer with HSV-1 vectors modified gC to remove the heparin binding domain and add a ligand for a specific protein on the cell surface, specifically a chimeric gC–erythropoietin (Laquerre et al., 1998a) or gC–His tag (Grandi et al., 2004). We reported the first targeted gene transfer to a specific type of neuron, nigrostriatal neurons, using HSV-1 vectors that contain either gC–glial cell line-derived neurotrophic factor (GDNF) or gC–brain-derived neurotrophic factor (BDNF) protein (Cao et al., 2008; Wang et al., 2005). Next, we developed a general strategy for targeting gene transfer to many different types of neurons, based on antibody-mediated targeted gene transfer (Cao et al., 2010). We constructed a chimeric gC—Staphylococcus A ZZ domain protein (gC–ZZ), and showed this protein is incorporated into HSV-1 particles and binds IgG. Complexes of these vector particles and an anti-NMDA receptor NR1 subunit antibody supported targeted gene transfer to NR1 subunit-containing neurons in rat neocortex, with long-term expression (Cao et al., 2010). However, because a majority of neocortical neurons contain NR1 subunits, the improvement in NR-1-neuron-specific expression was a relatively modest 15 to 20%, and this targeting specificity is of limited practical utility.

We now report on restricting recombinant expression to relatively rare types of neurons, of physiological significance, by using antibody-mediated targeted gene transfer in combination with a neuron-specific promoter. We developed targeted gene transfer to cells in rat postrhinal (POR) cortex that contain either NMDA NR2B or NR2A subunits. Further, the vector contained a modified neurofilament heave gene promoter that restricts expression to neurons (Zhang et al., 2000). Thus, this strategy restricts expression to neurons that contain either NMDA NR2B or NR2A subunits. Targeting increased NR2A-containing neuron-specific expression 50%, from 25 to 75%, demonstrating that targeting can support significant increases in specificity. The capability to selectively express genes in relatively rare types of forebrain neurons will benefit specific gene transfer studies.

2. Results

2.1. *Selective expression in NMDA NR2B subunit-containing neurons in rat POR cortex is supported by antibody-mediated targeted gene transfer to NR2B-containing cells with a vector containing a neuron-specific promoter*

Helper virus-free HSV-1 vector packaging was performed using a set of five cosmids that represent the HSV-1 genome, but lack the packaging site (contained in the **a** sequence) (Fraefel et al., 1996), and contain gC–ZZ for targeted gene transfer (Cao et al., 2010). Vector particle-antibody complexes were formed and purified using our established procedures (Cao et al., 2010). The resulting HSV-1 vector particles contain the vector, gC–ZZ+antibody for binding to specific types of neurons, and the other HSV-1 envelope glycoproteins required for subsequent cell entry (Spear and Longnecker, 2003).

The experimental design supports quantifying both targeted and untargeted gene transfer in POR cortex, in the same rats. Comparisons within the same rats are desirable because, given the heterogeneous cellular composition of neocortex, specific injections of HSV-1 vectors into neocortex likely support gene transfer to different neuron populations in different rats, complicating comparisons between rats. As HSV-1 particle entry occurs at a localized site on the cell surface, multiple HSV-1 virus particles can infect the same neuron (Javier et al.,

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